シナノグルミ(Juglans regia L.)のミクロ繁殖

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SHORT COMMUNICATION

Micropropagation of Shinano Walnut (*Juglans regia* L.)

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Summary

Micropropagation of three cultivars of Shinano walnut (*Juglans regia* L.) was performed by using DKW medium. Nodal segments (explants) produced new shoots on DKW medium supplemented with 5 μM BA and 0.05 μM IBA. When subcultured, shoots grew better on the above medium with agar than they did with Gelrite. There was a significant difference in the growth of shoots among the cultivars. To induce roots, IBA-treated shoots were transferred to a rooting medium, a Gelrite-solidified DKW medium with macroelements reduced to 1/4 strength, with or without vermiculite. The addition of vermiculite to the medium improved the rooting percentage and promoted the development of a good root system. All regenerated plantlets were successfully acclimatized.

Key Words: DKW medium, *Juglans regia*, micropropagation, Shinano walnut.

Introduction

Shinano walnut, mainly cultivated in Nagano prefecture, is believed to be a natural hybrid between *Juglans regia* L. (Persian walnut) and *J. regia* L. var. *orientis* Kitamura (Machida and Tanaka, 1958). Partly due to the difficulty in standard grafting, it is sometimes propagated by seedlings (Izaki and Maruhashi, 1989). Micropropagation may be a practical means of rapid, large-scale, clonal propagation of Shinano walnut cultivars selected by breeders (Machida and Tanaka, 1960). By using this method, we can avoid the expensive, time-consuming, and difficult process required for the grafting with an electric-heated hotbed (Koma, 1956). Self-rooted and micropropagated walnuts are planted in a 115 ha farm in Spain (Lopez, 2001). Successful micropropagation of Persian walnut was accomplished by several researchers (McGranahan et al., 1988; Leslie and McGranahan, 1992), after Driver and Kuniyuki (1984) developed the DKW medium for Paradox walnut rootstock (*J. hindsii* × *J. regia*). The objective of this study was to establish a micropropagation system for Shinano walnut cultivars by using DKW medium.

Materials and Methods

Shoots of 'Bansyun', 'Nan-an', and 'Seiko', were taken in May from 2- or 3-year-old grafted trees, planted in 8-liter pots in a greenhouse, and cut into 1-node cuttings, 2 to 3 cm long. After the leaves were removed, leaving 1 cm of the base of the petiole attached to the stem, the explants were rinsed for 30 min in running tap water, surface-sterilized for 20 min in 1% sodium hypochlorite solution, containing 0.1% Tween 20, and washed three times with sterile water. The explants were placed singly in a 100-ml conical beaker, containing 20 ml of DKW medium solidified with agar (Wako Pure Chemical Industries, Ltd.) or Gelrite (Monsanto Company) and supplemented with 5 μM BA and 0.05 μM IBA. The survival of explants and the number of expanded leaves on shoots were recorded after 30 days of culture.

Shoots from the explants were subcultured on the same DKW medium, solidified with agar at 1-month intervals for one year; then apical portions (containing apical buds with two expanded leaves, 3 to 5 mm long) of the shoots were imbedded on the same medium solidified with agar or Gelrite. The number of shoots, the height of the tallest shoot and the number of leaves were recorded after 30 days of culture.

Rooting was induced by placing the shoots, longer than 20 mm, in agar-solidified DKW medium with macroelements reduced to 1/4 strength and with 25 μM IBA for 5 days according to Jay-Allemand et al. (1992). After the root induction treatment, the shoots were transferred to the above rooting medium without IBA and with or without vermiculite (Nittai, Inc.). The vermiculite-containing Gelrite-solidified medium was prepared by pouring 40 ml of hot liquid medium containing Gelrite into a 100 ml conical beaker with 50 ml of vermiculite according to Jay-Allemand et al. (1992). After 30 days on culture, the shoots were removed from the medium, and the number of primary and secondary roots was recorded. The rooted shoots were potted and acclimatized according to Tetsumura et al. (1993).

All media contained 3% (w/v) sucrose and 0.2% Plant

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Preservative Mixture (Plant Cell Technology, Inc.) to reduce microbial contamination. The concentrations (w/v) of agar and Gelrite were 0.8% and 0.2%, respectively. Prior to autoclaving, the pH of the media was adjusted to 5.2. Cultures were maintained at 25 ℃ under 16-hr photoperiod with photon flux of 60 μmol·m^{-2}·sec^{-1} provided by cool-white fluorescent lamps. The root induction experiment was done in the dark.

Ten explants or shoots were used for each treatment, and each experiment replicated two or three times. All data were subjected to two-way (cultivar × medium) analysis of variance (ANOVA), and percentage of data was subjected to arcsin transformation before ANOVA.

Results and Discussion

After 30 days of culture on DKW medium, supplemented with 5 μM BA and 0.05 μM IBA and solidified with agar or Gelrite, 71% of the explants form three cultivars survived. A single shoot with leaves emerged on 54% of the explants with no significant difference among the cultivars or between agar and Gelrite (data not shown). For micropropagation of mature Persian walnut cultivars, McGranahan et al. (1988) found that frequent subculturing of the explants to fresh medium was needed to establish the explants and 18% of the explants were successfully subcultured. In this study, although microbial contamination damaged 27% of the explants, 37% of the explants were successfully subcultured.

When the apical portions of shoots were imbedded on the medium, the emerging shoots grew better on the agar-solidified medium than did those on the Gelrite-solidified medium (Table 1), on which some shoots showed hyperhydricity (vetrification). However, Leslie and McGranahan (1992) recommended the use of Gelrite, rather than agar, for walnut cultures, because agar-solidified DKW medium appears to have a detrimental effect. Ichimura and Oda (1998) reported that agar had growth-stimulating activity, which varied with the products. The agar used in this study might contain some growth stimulating substances and no inhibitors. There was a significant difference in the heights of tallest shoots among the cultivars (Table 1).

The addition of vermiculite to the rooting medium improved the rooting percentage and promoted the development of root system (Table 2). Jay-Allemand et al. (1992) reported similar results with *in vitro* cultured

Table 1. Effects of cultivar and solidifying agent on the number of shoots, the height of tallest shoot and the number of leaves of Shinano walnut after 30 days of culture on DKW medium supplemented with 5 μM BA and 0.05 μM IBA.

Cultivar	Solidifying agent	No. of shoots	Height of tallest shoot (mm)	No. of leaves
Bansyun	Agar	1.1	12.1	7.4
	Gelrite	1.0	9.0	6.5
Nan-an	Agar	1.7	18.6	8.2
	Gelrite	1.4	11.7	5.3
Seiko	Agar	1.6	19.7	8.1
	Gelrite	1.2	14.1	6.0
Significance				
Cultivar (C)		NS[z]	***	NS
Solidifying agent (SA)		*	*	*
C × SA		NS	NS	NS

[z] NS, *, ***: nonsignificant or significant at P<0.05 or 0.005, respectively.

Table 2. Effects of cultivar and substrate on the rooting percentage, the number of primary roots and the formation of secondary roots in Shinano walnut after 30 days of culture in the root development medium.

Cultivar	Substrate	Rooting(%)	No. of primary roots per rooted shoots	Secondary roots(%)[z]
Bansyun	Gelrite	3	1.0 ± 0.0[y]	0 ± 0[y]
	Gelrite+Vermiculite	13	1.2 ± 0.1	83 ± 14
Nan-an	Gelrite	0	-	-
	Gelrite+Vermiculite	47	2.1 ± 0.4	54 ± 3
Seiko	Gelrite	7	1.5 ± 0.0	0 ± 0
	Gelrite+Vermiculite	37	2.8 ± 0.7	58 ± 4
Significance				
Cultivar (C)		NS[x]		
Substrate (S)		***		
C × S		NS		

[z] No. of rooted shoots forming secondary roots/ No. of rooted shoots.

[y] Mean ± SE.

[x] NS, ***: nonsignificant or significant at P<0.005.

hybrid walnuts. In the vermiculite-containing Gelrite-solidified medium, half of the 'Nan-an' shoots rooted; none rooted in the Gelrite-solidified medium lacking vermiculite. Although the difference in rooting percentages among cultivars was not significant, that of 'Bansyun' was lowest even in the vermiculite treatment. In a preliminary trial, the use of the prerooting medium (Driver and Suttle, 1987) did not improve the rooting of Shinano walnut shoots. Although further modification to improve rooting is necessary, all plantlets were successfully acclimatized, irrespective of the substrates in the rooting medium .

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シナノグルミ (*Juglans regia* L.) のミクロ繁殖

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摘　要

DKW 培地を使用して，シナノグルミ (*Juglans regia* L.) 3品種のミクロ繁殖法を確立した．BA5 μM と IBA 0.05 μM を添加した DKW 培地上で，節間 (外植体) は新しいシュートを発生した．継代培養の際，ゲルライトで固化した培地よりも，寒天で固化した培地の方が，シュートの生長は優れた．またシュートの生長には有意な品種間差がみられた．IBA による発根誘導処理の後，多量要素を 4 分の 1 に減じ，ゲルライトで固化した DKW 培地にシュートを移植したが，この発根促進培地には，バーミキュライトを混入したものと未混入のもの作った．その結果，発根促進培地へのバーミキュライトの添加は，発根率を改善し，根系の発達を促すことが明らかとなった．発根したシュートはすべて順化に成功した．

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